Aquaculture Nutrition

Aquaculture Nutrition **2010 16**; 223–230 doi: 10.1111/j.1365-2095.2009.00654.x

Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in seawater and low-salinity water

M. RICHE & T.N. WILLIAMS

United States Department of Agriculture, Agricultural Research Service, Sustainable Marine Aquaculture Systems, Fort Pierce, FL, USA

Abstract

Two experiments were conducted with Florida pompano, *Trachinotus carolinus* L. at 3 and 28 g L^{-1} salinity to determine apparent crude protein digestibility (ACPD), energy digestibility (AED) and amino acid availability (AAAA) from soybean meal (SBM), soy protein isolate (SPI) and corn gluten meal (CGM). Mean AAAA was similar to ACPD. In fish adapted to 3 g L^{-1} salinity, they were 81.2% and 81.9% (CGM), 93.6% and 92.2% (SBM), 93.8% and 93.1% (SPI) for AAAA and ACPD respectively. In fish adapted to 28 g L^{-1}, they were 84.5% and 83.4% (CGM), 86.5% and 87.1% (SBM), and 83.4% and 85.0% (SPI) for AAAA and ACPD respectively. The AED was highest for SPI and lowest for SBM and inversely related to carbohydrate. The ACPD, AED and AAAA of soy products appeared to be lower in high salinity, whereas CGM was unaffected. The data suggest that SBM, SPI and CGM should be further evaluated as partial fishmeal replacements in Florida pompano diets. Application of the generated coefficients can be used to develop well-balanced, low-cost diets for Florida pompano reared in low salinity or seawater.

KEY WORDS: amino acid availability, digestible protein, plant-based proteins, pompano

Received 8 October 2008, accepted 16 December 2008

Correspondence: Marty Riche, USDA, ARS, 5600 US Hwy. 1, North, Fort Pierce, FL 39496, USA. E-mail: marty.riche@ars.usda.gov

Present address: Terhea N. Williams, Marine Bio-Resources, University of Maine, Rogers Hall, Orono, ME 04469, USA.

Introduction

Florida pompano, *Trachinotus carolinus* L. is a euryhaline species representing a small marine fishery in Florida with an estimated 227 000 kg total annual catch; however, because of its highly prized taste and texture it maintains a high market demand. Florida pompano tolerate a wide range of salinities, stress, readily consume pelleted rations, successfully breed in captivity, and are an excellent candidate for aquaculture (McMaster *et al.* 2004). There is increased interest in rearing euryhaline species such as Florida pompano in freshwater or low-salinity conditions. However, nutritionally balanced diets do not exist for Florida pompano presenting an obstacle to development of large-scale commercial production in low salinity.

High quality fish meal is the best source of protein for fish, particularly for carnivorous species. However, replacement of fish meal with alternative protein sources will increase sustainability and profitability (Glencross *et al.* 2007). In addition to palatability and anti-nutritional concerns, use of ingredients as alternatives to fishmeal is limited by unknown availability of nutrients. Apparent digestibility coefficients of feed ingredients exist for only a few fish species, but not Florida pompano. To develop low-cost, low-polluting diets that achieve maximum efficiency, nutrient requirements and nutrient availability from dietary ingredients must be determined to implement least-cost formulation of economical and balanced diets.

There is also evidence that salinity affects nutrient digestibility (Lall & Bishop 1976; MacLeod 1977; Dabrowski *et al.* 1986; Krogdahl *et al.* 2004). Digestibility in Golden-line seabream, *Sparus sarba* (Forsskal) was higher in low salinity relative to isosmotic or full-strength seawater (Woo & Kelly 1995). Similarly, protein digestibility in milkfish, *Chanos chanos* (Forsskal) was elevated in freshwater relative to saltwater (Ferraris *et al.* 1986). Zeitoun *et al.* (1973) also suggested that protein requirements of rainbow trout, *Oncorhynchus mykiss* (Walbaum) were higher with increasing salinity.

We hypothesized protein digestibility and amino acid (AA) availability would be different in low-salinity adapted Florida pompano than saltwater adapted Florida pompano. Therefore, the objective was to determine apparent digestibility of crude protein (CP), energy, and AA availability from soybean meal (SBM), soy protein isolate (SPI) and corn gluten meal (CGM) at both 3 and 28 g L^{-1} salinity representing the known range of salinity supporting normal growth of Florida pompano.

Materials and methods

Florida pompano broodstock were spawned at the USDA, Agricultural Research Service's Center for Reproduction and Larviculture, Fort Pierce, Florida, USA. Postlarval juveniles were reared at 28 °C and 30 g L^{-1} salinity. Fish were fed a commercial diet (EPAC-CW or IDL-CW; INVE Americas, Salt Lake City, UT, USA) until they were approximately 3 g in weight. Fish were subsequently transferred to a nursery system where they were held at 28 °C and 7 g L^{-1} salinity and fed a commercial trout diet (Silver Cup; Nelson & Sons, Inc., Murray, UT, USA) until they were approximately 75 g in weight at which time they were acclimated to 3 or 28 g L^{-1} salinity over a 1-week period.

Two simultaneous 4×4 Latin squares were set up to evaluate the three feed ingredients at 3 and 28 g L^{-1} salinity. Two 8750 L recirculating systems with sand, bead, cartridge and carbon filtration, and ultraviolet light sterilization were used. Both systems were maintained at 28 °C. Four 100-L tanks in each system with nominal flow rates of 3 L min^{-1} served as experimental units. Fish were maintained under a natural light cycle approximating 13 h light and 11 h dark.

A menhaden fish meal based formulation meeting the known protein and energy requirements for pompano served as the reference diet (Table 1). Solvent-extracted SBM (Rangen, Inc., Buhl, ID, USA), SPI (Archer Daniels Midland, Decatur, IL, USA) and CGM (Rangen, Inc.) were substituted at 300 g kg^{-1} for 300 g kg^{-1} of the reference diet utilizing a modified diet replacement method. All diets incorporated yttrium oxide (Y_2O_3) at 5 g kg^{-1} of the diet as an inert marker. Feed ingredients were ground via hammermill (Prater Industries, Inc., Chicago, IL, USA) to pass a 250 micron screen. Dry feed ingredients were mixed in a V-mixer (Patterson-Kelley, East Stroudsburg, PA, USA). Following addition of water and oil, complete diets were cold extruded and dried at 60 °C for 24 h. Pelleted diets were stored at −20 °C until fed.

Twenty and 15 fish each, were stocked into 28 and 3 g L^{-1} salinity experimental units respectively. Fish were fed a commercial diet and allowed a 4-day acclimation to the new environment. At initiation of the experiment, fish were switched to their assigned experimental diet and fed 4.7% body weight per day divided between a morning and afternoon feeding. Faecal samples were collected on day 5 and day 7 of being fed the experimental diets. Faecal samples were collected 3–4 h following the morning feeding on day of collection.

Prior to faecal collection, fish were anaesthetized with 75 mg L^{-1} tricaine methanesulphonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). Upon induction of stage IV anaesthesia, the area around the anus was dried with a towel and faecal samples collected by gentle expression of the lower gastrointestinal tract (Austreng 1978). Immature fish were used and care was taken not to contaminate samples with urine. Following collection, fish were resuscitated and placed back into the experimental unit. Faeces collected

Table 1 Reference and test diets used to determine digestibility of crude protein, energy and amino acid availability from soybean meal, soy protein isolate, and corn gluten meal in Florida pompano *Trachinotus carolinus*

Ingredient (g kg^{-1} dry diet)	Reference diet	Test diets
Test ingredient	0.0	300.0
Menhaden meal (low temperature)[1]	338.5	237.0
Soybean meal (solvent extracted)[2]	221.0	154.7
Corn gluten meal[2]	68.0	47.6
Porcine blood meal (spray dried)[2]	30.0	21.0
Fish solubles (dehydrated)[3]	60.0	42.0
Shrimp meal[2]	50.0	35.0
Dextrin (type-II from corn)[4]	22.0	15.4
Menhaden oil (stabilized)[5]	139.0	97.3
Sipernat 50[6]	10.0	7.0
Mineral premix[7]	15.0	10.5
Vitamin premix[8]	5.0	3.5
Lecithin[9]	1.0	0.7
Ascorbyl-2-monophosphate[8]	0.5	0.4
α-Cellulose[10]	15.0	9.0
Carboxymethyl cellulose[10]	20.0	14.0
Yttrium oxide[10]	5.0	5.0

[1] Special Select™, Omega Protein, Inc., Houston, TX, USA.
[2] Rangen Inc., Buhl, ID, USA.
[3] International Proteins Corp., Minneapolis, MN, USA.
[4] MP Biomedicals, Solon, OH, USA.
[5] Alkali refined and stabilized with 500 ppm ethoxyquin, Omega Protein, Inc., Hammond. LA, USA.
[6] Degussa Corp., Parsippany, NJ, USA.
[7] Mineral premix contained the following (g kg^{-1} premix): $CaHPO_4$, 350.0; $CaSO_4 \cdot 2H_20$, 100.0; KH_2PO_4, 200.0; $MgSO_4 \cdot 7H_20$, 84.0; $FeSO_4 \cdot 7H_2O$, 16.0; $ZnSO_4 \cdot 7H_2O$, 3.0; $MnSO_4 \cdot H_2O$, 2.0; $CuCl_2 \cdot 2H_20$, 1.0; KF, 0.23; KI, 0.1; $NaMoO_4 \cdot 2H_2O$, 0.05; $CoCl_2 \cdot 6H_2O$, 0.02; Na_2SeO_3, 0.01.
[8] Roche Vitamins Inc, Parsippany, NJ, USA.
[9] USB, Cleveland, OH, USA.
[10] Sigma-Aldrich, St. Louis, MO, USA.

on both day 5 and 7 were pooled into one sample. Diets were reassigned to the experimental units and procedures were repeated until all four experimental units received each of the four diets (4 weeks).

Feed and pooled faecal samples were analysed for yttrium (Y), nitrogen (N), gross energy (GE) and AA. Proximate composition of reference and test diets was determined and test ingredients were analysed for each ingredient's contribution of nutrients to the test diet (Table 2). Coefficients were calculated as the ratio of nutrient and marker in feed and faeces (Maynard & Loosli 1969) and adjusted for nutrient concentration (Forster 1999).

Test ingredients, feed and faecal samples were lyophilized to a constant weight and stored at −80 °C until analysis. Nitrogen was determined following combustion (TruSpec N-elemental analyser; Leco Corp., St. Joseph, MI, USA) and CP calculated as $N \times 6.25$. GE was determined by adiabatic bomb calorimetry (Parr 1266; Parr Instruments Co., Moline, IL, USA). Ash was determined following incineration at 600 °C for 2 h (AOAC 2002). Crude lipid was determined gravimetrically following chloroform : methanol extraction (Bligh & Dyer 1959) in a Soxhlet apparatus. Crude fibre was determined by a commercial laboratory (Barrow-Agee Laboratories, Memphis, TN, USA).

Amino acids were analysed by a commercial laboratory (Midwest Laboratories, Inc., Omaha, NE, USA). Briefly, samples were hydrolyzed with 6 N HCl at 110 °C for 24 h. A separate aliquot was analysed for cysteine (Cys) and methionine (Met) following performic acid oxidation to cysteic acid and methionine sulphone. Amino acids were separated using a C-18 reverse phase HPLC column and quantified with a photodiode array detector following postcolumn derivatization with ninhydrin.

Table 2 Analysed composition (g kg^{-1}) of test ingredients and experimental diets fed to Florida pompano *Trachinotus carolinus*

	Test ingredient						
International feed no.	CGM[1] 5-28-242	SBM[2] 5-04-612	SPI[3] –	Reference diet	CGM diet	SBM diet	SPI diet
Proximate components							
Dry matter	917.0	894.0	915.0	947.0	884.0	882.0	926.0
Crude protein	653.0	474.0	885.0	523.0	542.0	498.0	628.0
Crude lipid	22.0	11.0	2.0	160.0	118.0	107.0	108.0
Ash	26.0	57.0	39.0	144.0	103.0	123.0	110.0
Fibre	8.0	33.0	2.0	34.0	27.0	35.0	24.0
NFE[4]	214.0	342.0	0.0	86.0	94.0	119.0	56.0
Gross energy (kJ g^{-1})	20.9	17.4	20.9	21.0	21.7	20.8	21.9
Indispensable amino acids							
Arginine	21.1	34.9	60.3	26.5	22.6	27.6	37.6
Histidine	19.8	14.4	17.0	13.5	12.5	12.7	15.9
Isoleucine	24.2	19.7	42.2	20.5	19.3	18.4	27.3
Leucine	84.8	40.4	75.0	46.7	60.9	39.4	52.5
Lysine	10.0	30.6	53.6	32.6	25.3	31.4	37.6
Methionine	24.4	10.3	10.9	13.5	14.3	12.2	12.7
Phenylalanine	45.5	25.5	46.5	22.9	27.3	21.9	29.4
Threonine	23.9	19.9	35.1	20.4	20.2	19.5	24.6
Valine	29.2	21.9	41.3	27.5	26.5	21.0	32.9
Dispensable amino acids							
Alanine	66.6	21.9	51.5	40.4	49.8	35.9	46.4
Asx[5]	43.2	58.8	112.0	50.1	45.5	51.2	67.7
Cysteine	21.5	10.4	08.4	14.5	11.9	13.2	11.2
Glx[6]	170.3	99.6	162.0	74.6	89.8	76.9	101.0
Glycine	18.9	21.9	38.8	30.8	25.7	26.6	32.5
Proline	55.1	25.5	44.5	24.5	33.4	24.1	29.9
Serine	41.0	28.1	50.5	23.2	25.7	23.4	30.6
Tyrosine	35.8	18.1	32.2	16.2	20.8	15.7	20.6

[1] Corn gluten meal, Rangen, Inc., Buhl, ID, USA.
[2] Dehulled, solvent-extracted soybean meal; Rangen, Inc., Buhl, ID, USA.
[3] Soy protein isolate, Pro-Fam®, Archer Daniels Midland, Decatur, IL, USA.
[4] Nitrogen-free extract (100 − moisture − crude protein − crude lipid − ash − fibre).
[5] Aspartic acid + asparagine.
[6] Glutamic acid + glutamine.

Differences in apparent nutrient availability were analysed using the model statement for a Latin square design:

$$Y_{ijk} = \mu + I_i + \text{column}_j + \text{row}_k + \varepsilon_{ijk},$$

where I represents the main effect of test ingredient, column represents variation due to tank, and row represents variation due to week. Analysis was performed using the general linear model procedure of SAS with software package version 9.1 (SAS Institute, Cary, NC, USA). Residuals were analysed to evaluate normality of distribution and homogeneity of variance. Where main effect differences were detected pair-wise contrasts between the three ingredients were evaluated. Significance was reported at $P < 0.05$ unless otherwise stated. Where analysis indicated row or column effects in 3 g L^{-1} salinity (alanine) or 28 g L^{-1} (glutamic acid + glutamine) no further analysis was conducted as row and column both represent restrictions on randomization making the F-test questionable. Regression analysis was performed with test ingredient protein and energy as independent variables and apparent energy digestibility (AED) as the dependent variable.

Results

Total ammonia-nitrogen ranged from 0.00 to 0.21 mg L^{-1} and 0.01 to 0.17 mg L^{-1} for the low-salinity and saltwater systems, respectively. Nitrite-nitrogen was 0.04–5.01 and 0.04–0.56 mg L^{-1} for the low-salinity and saltwater systems, respectively. The pH and alkalinity ranged from 6.92 to 8.08 mg L^{-1} and 138 to 190 mg L^{-1} as $CaCO_3$ at 3 g L^{-1} salinity and from 6.62 to 7.95 mg L^{-1} and 86 to 139 mg L^{-1} as $CaCO_3$ at 28 g L^{-1} salinity. Values were within acceptable ranges for Florida pompano (Watanabe 1995; Weirich & Riche 2006). No mortalities occurred during the experiment.

Apparent crude protein digestibility (ACPD) was significantly higher in the soy products than CGM at low salinity, but not in sea water where no differences were detected (Table 3). The AED was higher from SPI than CGM and SBM at low salinity. Despite a decrease in AED of the soy products at 28 g L^{-1}, the coefficient for SPI remained higher than SBM, but not CGM.

Insufficient faeces necessitated reporting apparent Met and Cys availability on either two or three samples. Therefore, statistical analysis was not performed on these two AA. Significant differences in apparent amino acid availability (AAAA) were detected for phenylalanine (Phe) and glutamic acid + glutamine (Glx) at 3 g L^{-1} salinity (Table 4). No other differences were detected at low salinity. Although not statistically different, the overall pattern suggests that AAAA appears higher from soy products than CGM at low salinity in agreement with ACPD. The availability of Met approached 100% for all ingredients. Overall mean AAAA was similar to ACPD for all test ingredients, they were 81.2% and 81.9% (CGM), 93.6% and 92.2% (SBM), 93.8% and 93.1% (SPI) for AAAA and ACPD respectively.

Table 3 Mean (SEM, n = 4) apparent crude protein (ACPD) and energy (AED) digestibility coefficients (%) for soybean meal, soy protein isolate and corn gluten meal fed to Florida pompano *Trachinotus carolinus* adapted to 3 or 28 g L^{-1} salinity

	ACPD		AED	
Test ingredient	3 g L^{-1}	28 g L^{-1}	3 g L^{-1}	28 g L^{-1}
Reference diet	72.8 (0.5)	74.7 (1.1)	71.3 (1.2)	72.3 (0.7)
Corn gluten meal	81.9 (4.2)[b]	83.4 (2.9)[a]	77.4 (4.2)[b]	77.4 (3.4)[a]
Soybean meal	92.2 (2.0)[a]	87.1 (3.6)[a]	70.5 (6.5)[b]	62.2 (4.0)[b]
Soy protein isolate	93.1 (1.9)[a]	85.0 (3.5)[a]	93.4 (2.5)[a]	78.1 (4.1)[a]

Mean values within a column having different superscripts were significantly different ($P < 0.05$).

Table 4 Mean (SEM; n = 4) apparent amino acid availability (AAAA) coefficients (%) for soybean meal (SBM), soy protein isolate (SPI) and corn gluten meal (CGM) in Florida pompano *Trachinotus carolinus* adapted to 3 g L^{-1} salinity

Amino acids	Reference diet	CGM	SBM	SPI
Indispensable				
Arginine	83.3 (1.0)	73.5 (23.3)	102.0 (7.4)	95.3 (1.4)
Histidine	80.3 (0.6)	76.8 (6.1)	103.3 (9.0)	92.5 (4.5)
Isoleucine	80.4 (1.2)	68.1 (10.4)	91.8 (11.7)	96.4 (1.7)
Leucine	86.1 (0.3)	88.1 (4.3)	92.1 (1.1)	94.7 (1.0)
Lysine	81.9 (1.1)	76.2 (17.9)	100.0 (4.7)	94.1 (2.6)
Methionine[1]	83.0 (1.8)	100.0 (2.8)	110.1 (6.0)	105.7 (8.1)
Phenylalanine	84.0 (0.5)	83.2 (6.7)[b]	97.0 (3.5)[a]	95.1 (2.7)[a]
Threonine	75.3 (1.0)	81.0 (9.4)	92.3 (6.0)	89.9 (6.4)
Valine	82.2 (2.0)	81.6 (7.9)	85.6 (8.5)	98.6 (3.1)
Dispensable				
Alanine	81.5 (1.3)	91.5 (5.0)	89.7 (10.9)	96.4 (2.0)
Asx[2]	73.9 (0.3)	79.3 (7.1)	87.7 (3.8)	90.9 (4.1)
Cysteine[1]	84.5 (0.5)	67.8 (10.9)	91.5 (3.3)	82.8 (5.2)
Glx[3]	80.0 (0.6)	86.5 (3.7)[b]	94.0 (3.1)[a]	93.8 (3.1)[a]
Glycine	71.8 (1.4)	72.8 (9.8)	71.7 (12.3)	88.4 (2.7)
Proline	73.5 (0.7)	84.2 (3.5)	88.9 (2.5)	93.1 (3.4)
Serine	79.3 (1.3)	84.9 (7.3)	94.5 (4.4)	93.6 (3.5)
Tyrosine	83.1 (1.0)	84.8 (9.2)	99.4 (7.1)	92.9 (3.4)
Overall mean AAAA	80.2 (1.0)	81.2 (2.0)	93.6 (2.1)	93.8 (1.2)

Different superscripts across a row indicate significant differences between ingredients tested ($P < 0.05$).
[1] Not statistically evaluated due to insufficient material for suitable replication (n = 2).
[2] Aspartic acid + asparagine.
[3] Glutamic acid + glutamine.

Table 5 Mean (SEM; n = 4) apparent amino acid availability (AAAA) coefficients (%) for soybean meal (SBM), soy protein isolate (SPI) and corn gluten meal (CGM) in Florida pompano *Trachinotus carolinus* adapted to 28 g L^{-1} salinity

Amino acids	Reference diet	CGM	SBM	SPI
Indispensable				
Arginine	87.5 (0.6)	89.0 (3.3)	78.1 (10.2)	79.4 (16.8)
Histidine	81.1 (1.1)	84.4 (5.5)	88.7 (7.7)	83.6 (4.6)
Isoleucine	81.6 (1.6)	79.6 (5.4)	84.6 (17.9)	91.9 (5.9)
Leucine	87.7 (0.7)	92.0 (1.4)	85.6 (7.1)	85.4 (5.6)
Lysine	83.0 (0.4)	77.4 (7.9)^{b}	95.6 (2.1)^{a}	83.8 (4.2)^{ab}
Methionine[1]	87.9 (0.3)	92.9 (1.8)	93.9 (6.0)	90.2 (4.5)
Phenylalanine	85.6 (0.4)	90.2 (0.9)	79.6 (11.8)	85.2 (3.5)
Threonine	73.2 (3.5)	87.6 (5.3)	105.3 (4.8)	87.7 (9.3)
Valine	86.2 (0.6)	84.6 (0.8)^{ab}	75.7 (5.3)^{b}	88.1 (5.0)^{a}
Dispensable				
Alanine	84.9 (0.7)	88.7 (1.9)	80.9 (10.9)	81.6 (5.3)
Asx[2]	73.7 (2.4)	79.0 (6.2)	98.7 (15.4)	80.6 (5.6)
Cysteine[1]	85.3 (1.0)	68.3 (1.7)	73.7 (2.4)	51.9 (8.1)
Glx[3]	81.9 (0.4)	86.8 (2.8)	87.9 (3.0)	86.2 (3.5)
Glycine	72.5 (1.5)	68.8 (9.6)	86.1 (16.4)	77.0 (7.3)
Proline	77.6 (0.4)	84.3 (3.1)	79.5 (8.2)	84.2 (4.1)
Serine	75.7 (3.8)	93.1 (5.5)	105.7 (4.8)	92.4 (6.8)
Tyrosine	85.5 (0.5)	89.3 (1.2)	70.6 (19.2)	88.5 (4.2)
Overall mean AAAA	81.8 (1.3)	84.5 (1.8)	86.5 (2.5)	83.4 (2.2)

Different superscripts across a row indicate significant differences between ingredients tested ($P < 0.05$).

[1] Not statistically evaluated due to insufficient material for suitable replication (CGM, n = 3; SBM, n = 3; SPI, n = 2).

[2] Aspartic acid + asparagine.

[3] Glutamic acid + glutamine.

Significant differences in AAAA were detected for lysine (Lys) and valine (Val) at 28 g L^{-1} (Table 5). Availability of Lys was higher from SBM (95.6%) than CGM (77.4%), and neither was different from SPI (83.8%). Apparent availability of Val was higher from SPI (88.1%) than SBM (75.7%), and neither was different from CGM (84.6%). No other differences were detected at 28 g L^{-1} salinity. As with low-salinity treatments, overall mean AAAA was similar to ACPD for all test ingredients. They were 84.5% and 83.4% (CGM), 86.5% and 87.1% (SBM), and 83.4% and 85.0% (SPI) for AAAA and ACPD respectively.

Discussion

Apparent digestibility of CP was high for all test ingredients regardless of salinity, particularly relative to the reference diet. The high ACPDs suggest a potential for these plant proteins as partial replacements for fish meal in Florida pompano diets. Apparent digestibilities of CP and GE from the reference diet were lower than reported for some marine species fed compounded diets (Santinha *et al*. 1999; Peres & Oliva-Teles 1999; Sá *et al*. 2006). The reason is unclear; however, the values in this study are similar to previously reported values (75.8% and 73.3% for ACPD and AED respectively) for juvenile Florida pompano fed the same diet formulation (Riche, new characters, 2009).

Poor digestibility is one reason attributed to low feed efficiency (FE) in Florida pompano (Tatum 1973; McMaster 1988; Lazo *et al*. 1998; Weirich *et al*. 2006). However, SBM digestibility and AAAA at 3 g L^{-1} salinity were similar to that observed in yellowfin sea bream, *Acanthopagrus latus* (Houttuyn) (Wu *et al*. 2006) and Atlantic cod, *Gadus morhu*a L. (Tibbetts *et al*. 2006). Also, ACPD for SBM at 28 g L^{-1} salinity was the same as reported for gilthead seabream, *Sparus aurata* L. (Lupatsch *et al*. 1997). Although ACPD for SBM was similar to that reported for haddock, *Melanogrammus aeglefinus* L. (92.2%) and Cobia, *Rachycentron canadum* L. (92.8%), AED was approximately 18–20% lower in Florida pompano than haddock or cobia (Tibbetts *et al*. 2004; Zhou *et al*. 2004). Low apparent digestible energy values from SBM were also reported in European seabass, *Dicentrarchus labrax* L. (da Silva & Oliva-Teles 1998) and red drum, *Sciaenops ocellatus* L. (Gaylord & Gatlin 1996).

De Silva & Perera (1984) suggested that lower protein digestibility occurs in diets with higher protein. However, in this study no difference in protein digestibility between soy products was detected at either salinity despite 130 g kg^{-1} higher protein in the SPI diet. Conversely, in this study AED was directly proportional to dietary protein (r^2 = 1.00) and inversely proportional to dietary nitrogen free extract (NFE; r^2 = 0.99). Utilization of plant starch is limited in fish, particularly carnivores. Digestible energy tends to be negatively correlated to dietary carbohydrate and positively correlated to dietary protein and lipid (Sullivan & Reigh 1995). Carbohydrate digestibility in Florida pompano is about 50% (Williams *et al*. 1985) underscoring its limited availability and impact on energy digestibility.

Florida pompano have short digestive tracts. Intestinal transit time for a fish meal/SBM diet was reported as 3 h in seawater at 29–31 °C (Williams *et al*. 1985). This was later confirmed using the same dietary formulation serving as the reference diet in this study (Riche, new characters, 2009). The short transit may result in limited enzymatic contact time attenuating digestion and absorption of nutrients, possibly causing the poor FE reported for Florida pompano.

Faecal stripping was initiated 3 h postprandially. Consistent results with previous trials (Riche, new characters, 2009) coupled with the small SEM of coefficients in the reference diet suggests that this was appropriate for the reference diet. However, the high SEM of coefficients associated with the test ingredients, particularly AAAA coefficients for SBM and CGM suggests incomplete digestion or possible interactive effects. Composition, chemical, and physical characteristics of feed can affect both. Also, faecal collection method affects variability of availability values, with greater variability observed using faecal stripping (Yamamoto *et al.* 1997).

Digestibility coefficients are also generally lower using intestinal stripping relative to other methods (Hajen *et al.* 1993; Yamamoto *et al.* 1997). However, Glencross *et al.* (2005) demonstrated feed ingredients high in carbohydrates, such as SBM and CGM, affect faecal pellet integrity and suggested that stripping is the preferred faecal collection method for plant protein digestibility trials. Moreover, this method obviates diluting nutrient concentrations by external saltwater contamination of faeces.

Digestibility coefficients for SBM and SPI reported for Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) were much lower than for Florida pompano (Hajen *et al.* 1993). Conversely, energy and N digestibility of SPI in rainbow trout (Glencross *et al.* 2005) and Atlantic cod (Tibbetts *et al.* 2004) were higher than for pompano, while N digestibility for SBM was the same. The significant difference observed in AED between SBM and SPI was also observed in rainbow trout and Atlantic salmon, *Salmo salar* L. (Glencross *et al.* 2004), again supporting the negative effect of carbohydrates on digestible energy in carnivorous species.

Protein digestibility of the soy products was higher than CGM at low salinity, but not at 28 g L^{-1}. Energy digestibility of CGM was similar in haddock, but ACPD in haddock was approximately 10% higher (Tibbetts *et al.* 2004). Also, the energy digestibility coefficient of non-extruded CGM in rainbow trout was similar to that reported here, but increased substantially following extrusion (Cheng & Hardy 2003). It is likely extrusion processing would increase ADE of CGM in Florida pompano as well.

The AAAA from SBM in Florida pompano was similar to yellowfin seabream, *Sparus latus* (Houttuyn) with the exception of Lys and Phe availability being higher, and Val lower in pompano (Wu *et al.* 2006). In cobia, AAAA from SBM was similar to Florida pompano, but that from CGM was higher ranging from 93.2% to 96.9% (Zhou *et al.* 2004). Overall AAAA of SBM and SPI reflected CP digestibility as reported elsewhere (Yamamoto *et al.* 1997; Allan *et al.* 2000; Zhou *et al.* 2004).

The AAAA from CGM was 5.7–16.3% lower relative to Australian silver perch, *Bidyanus bidyanus* (Mitchell) for all indispensable AA except Met (Allan *et al.* 2000). They were also substantially lower than in rainbow trout where all AAAA were >95% (Yamamoto *et al.* 1997). Pompano fed a CGM based diet supplemented with AA to match their whole body AA profile exhibited only 60% of the weight gain of pompano fed a menhaden meal based diet with the same AA profile (Riche; unpublished data). Results from this study suggest that poor weight gain previously observed was due in part to lower AA availability from CGM.

Apparent availability of Met was high for all test ingredients, as it was in cobia (Zhou *et al.* 2004). The Met availability from test ingredients evaluated in low salinity was 100–110%, suggesting enhanced availability from the other protein sources used in the test diets. However, caution should be exercised in interpreting the Met values as insufficient material in some cases limited the number of samples for estimating means.

Significantly, lower apparent Lys availability was observed from CGM than SBM at the higher salinity ($P < 0.05$) and appeared to be lower than both soy products at low salinity. This is similar to that reported for Australian silver perch (Allan *et al.* 2000), red sea bream, *Pagrus major* (Temminck & Schlegel) (Yamamoto *et al.* 1998), and yellowtail, *Seriola quinqueradiata* (Temminck & Schlegel) (Masumoto *et al.* 1996), but the opposite of cobia (Zhou *et al.* 2004) and Atlantic salmon (Anderson *et al.* 1992). Lower Lys availability from CGM relative to the soy products may be an artefact of lower Lys in CGM. Analysis of test ingredients indicated Lys was 53.6, 30.6 and 10.0 g kg^{-1} dry matter for SPI, SBM and CGM respectively. At low dietary Lys, endogenous sources account for more of the recovered Lys masking true availability and depressing apparent availability. The 10% increase in true Lys availability over apparent Lys availability from CGM in red sea bream (Yamamoto *et al.* 1998) and yellowtail (Masumoto *et al.* 1996) support this hypothesis.

The low CGM coefficients and high variability for Arg (SEM of 23.3%) and Lys (SEM of 17.9%) in the low salinity treatment are attributable to high recovery of these AA in one faecal sample resulting in AAAA for that replicate of 6.5% and 29.1% for Arg and Lys respectively. Removal of that sample from consideration would have resulted in coefficients of 95.8% and 91.9% for Arg and Lys, respectively, which are similar to the other ingredients. Although residuals of the coefficients tested as outliers (Snedecor & Cochran 1967), the coefficients were not removed from analysis because row and column effects could not be ruled out. Moreover, it is possible the coefficients could represent true variability in AAAA for a marine species held at low salinity.

Although the experimental design precludes statistical analysis of test ingredients between the two salinities, the trend was towards higher ACPD and AED from SBM and SPI for pompano reared in low salinity water relative to seawater. This could result in lower FE in saltwater and suggests that dietary protein may need to be higher for production in saltwater as reported for other species (Zeitoun *et al*. 1973; Lall & Bishop 1976). The data suggest that further research is warranted to determine if digestibility values are lower in a seawater environment.

In summary, the ACPD of SBM and SPI were >90% in low salinity, and significantly higher than CGM. However, no differences in ACPD could be detected between the three ingredients in seawater. As the ACPD coefficient for CGM was similar between the two salinities it appears protein digestibility of the soy products may be lower in seawater than freshwater, although this could not be tested. The AED for the three test ingredients exhibited a parallel response to salinity as the ACPD. The AED of SBM was significantly lower than SPI and was likely due to the CP/NFE ratio as there was a positive linear relationship ($r^2 = 1.00$) with protein and inverse relationship ($r^2 = 0.99$) with NFE. The overall AAAA from the test ingredients was similar to the ACPD coefficients and suggests that SBM, SPI and CGM should be further evaluated as partial fishmeal replacements in Florida pompano diets. Application of the protein, energy and AA coefficients for SBM, SPI, and CGM generated in this study can be used to develop well-balanced, low-cost diets for Florida pompano reared in low salinity or in seawater addressing one of the obstacles to large-scale commercial production of this species.

Acknowledgements

The authors acknowledge David I. Haley and Patrick L. Tracy for their skilful technical assistance in sample collection, preparation and processing. We would also like to express gratitude to Dr T. Gibson Gaylord, Dr Jon Amberg and Dr Hector Acosta-Salmon for critical review and advice on preparation of this manuscript. The authors acknowledge Rangen, Inc. Buhl, Idaho for generously donating the plant-based proteins. This work was funded in part by the Link Foundation. Additional funding was provided by the USDA/Agricultural Research Service Project No. 6225-63000-007-00D. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. All programmes and services of the US Department of Agriculture are offered on a non-discriminatory basis without regard to race, colour, national origin, religion, sex, marital status, or handicap.

References

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. & Warner-Smith, R. (2000) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, **186,** 293–310.

Anderson, J.S., Lall, S.P., Anderson, D.M. & Chandrasoma, J. (1992) Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. *Aquaculture*, **108,** 111–124.

AOAC (Association of Official Analytical Chemists) (2002) *Official Methods of Analysis of the Association of Analytical Chemists*, 17th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Austreng, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, **13,** 265–272.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys*., **37,** 911–917.

Cheng, Z.J. & Hardy, R.W. (2003) Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquacult. Nutr*., **9,** 77–83.

Dabrowski, K., Leray, C., Nonnotte, G. & Colin, D.A. (1986) Protein digestion and ion concentrations in rainbow trout (*Salmo gairdneri* Rich.) digestive tract in sea- and fresh water. *Comp. Biochem. Physiol*., **83A,** 27–39.

De Silva, S.S. & Perera, M.K. (1984) Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on variability in daily digestibility. *Aquaculture*, **38,** 293–306.

Ferraris, R.P., Catacutan, M.R., Mabelin, R.L. & Jazul, A.P. (1986) Digestibility in milkfish, *Chanos chanos* (Forsskal): effects of protein source, fish size and salinity. *Aquaculture*, **59,** 93–105.

Forster, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquacult. Nutr*., **5,** 143–145.

Gaylord, T.G. & Gatlin, D.M., III (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture*, **139,** 303–314.

Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Maas, R. & Sipsas, S. (2004) A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **237,** 333–346.

Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R. & Sipsas, S. (2005) Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. *Aquaculture*, **245,** 211–220.

Glencross, B.D., Booth, M. & Allan, G.L. (2007) A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquacult. Nutr*., **13,** 17–34.

Hajen, W.E., Higgs, D.A., Beames, R.M. & Dosanjh, B.S. (1993) Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in sea water. Measurement of digestibility. *Aquaculture*, **112,** 333–348.

Krogdahl, Å., Sundby, A. & Olli, J.J. (2004) Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. *Aquaculture*, **229,** 335–360.

Lall, S.P. & Bishop, F.J. (1976) Studies on the nutrient requirements of rainbow trout, *Salmo gairdneri*, grown in sea water and fresh water. In: *Advances in Aquaculture: FAO Technical Conference on Aquaculture, Kyoto, Japan. 26 May–2 June 1976*. (Pillay, T.V.R. & Dill, W.A. eds), pp. 580–584. Fishing News Books, Farnham, England.

Lazo, J.P., Davis, D.A. & Arnold, C.R. (1998) The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). *Aquaculture*, **169,** 225–232.

Lupatsch, I., Kissil, G.W., Sklan, D. & Pfeffer, E. (1997) Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. *Aquacult. Nutr*., **3,** 81–89.

MacLeod, M.G. (1977) Effects of salinity on food intake, absorption and conversion in the rainbow trout *Salmo gairdneri*. *Mar. Biol*., **43,** 93–102.

Masumoto, T., Ruchimat, T., Ito, Y., Hosokawa, H. & Shimeno, S. (1996) Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). *Aquaculture*, **146,** 109–119.

Maynard, L.A. & Loosli, J.K. (1969) *Animal Nutrition*, 5th edn. McGraw-Hill Book Company, New York, NY, USA, 613 pp.

McMaster, M.F. (1988) Pompano aquaculture: past success and present opportunities. *Aquacult. Mag*., **14,** 28–34.

McMaster, M.F., Kloty, T.C. & Coburn, J.F. (2004) Pompano mariculture – 2004. *Aquacult. Mag*., **30,** 25–29.

Peres, H. & Oliva-Teles, A. (1999) Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture*, **179,** 325–334.

Riche, M. (2009) Evaluation of digestible energy and protein for growth and nitrogen retention in juvenile Florida pompano, *Trachinotus carolinus*. *J. World Aquacult. Soc*., **40,** 45–57.

Sá, R., Pousão-Ferreira, P. & Oliva-Teles, A. (2006) Effect of dietary protein and lipid levels on growth and feed utilization of white sea bream (*Diplodus sargus*) juveniles. *Aquacult. Nutr*., **12,** 310–321.

Santinha, P.J.M., Medale, F., Corraze, G. & Gomes, E.F.S. (1999) Effects of the dietary protein: lipid ratio on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). *Aquacult. Nutr*., **5,** 147–156.

da Silva, J.G. & Oliva-Teles, A. (1998) Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. *Aqua. Living Resour*., **11,** 187–191.

Snedecor, G.W. & Cochran, W.G. (1967) *Statistical Methods*, 6th edn. The Iowa State University Press, Ames, IA, USA, 593 pp.

Sullivan, J.A. & Reigh, R.C. (1995) Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilis* ♀ × *Morone chrysops* ♂). *Aquaculture*, **138,** 313–322.

Tatum, W.M. (1973) Comparative growth of pompano (*Trachinotus carolinus*) in suspended cages receiving diets of a floating trout chow with those receiving a mixture of 50% trout chow and 50% sinking ration. In: *Proceedings of the 4th Annual Meeting of the World Mariculture Society* (Avault, J.W. & Boudreaux, E. eds), pp. 125–141. Louisiana State University, Baton Rouge, LA, USA.

Tibbetts, S.M., Milley, J.E. & Lall, S.P. (2006) Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture*, **261,** 1314–1327.

Tibbetts, S.M., Lall, S.P. & Milley, J.E. (2004) Apparent digestibility of common feed ingredients by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquac. Res*., **35,** 643–651.

Watanabe, W.O.. (1995) Aquaculture of the Florida pompano and other jacks (Family Carangidae) in the Western Atlantic, Gulf of Mexico, and Caribbean basin: status and potential. In: *Culture of High-value Marine Fishes* (Main, K.L. & Rosenfeld, C. eds), pp. 185–205. Oceanic Institute, Honolulu, HI, USA.

Weirich, C.R. & Riche, M. (2006) Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L. to ammonia and nitrite at various salinities. *Aquac. Res*., **37,** 855–861.

Weirich, C.R., Groat, D.R., Reigh, R.C., Chesney, E.J. & Malone, R.F. (2006) Effect of feeding strategies on production characteristics and body compoasition of Florida pompano reared in marine recirculating systems. *N. Am. J. Aquacult*., **68,** 330–338.

Williams, S., Lovell, R.T. & Hawke, J.P. (1985) Value of menhaden oil in diets of Florida pompano. *Prog. Fish Cult*., **47,** 159–165.

Woo, N.Y.S. & Kelly, P.S. (1995) Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. *Aquaculture*, **135,** 229–238.

Wu, X., Liu, Y., Tian, L., Mai, K. & Yang, H. (2006) Apparent digestibility coefficients of selected feed ingredients for yellowfin seabream, *Sparus latus*. *J. World Aquacult. Soc*., **37,** 237–245.

Yamamoto, T., Ikeda, K., Unuma, T. & Akiyama, T. (1997) Apparent availabilities of amino acids and minerals from several protein sources for fingerling rainbow trout. *Fish. Sci*., **63,** 995–1001.

Yamamoto, T., Akimoto, A., Kishi, S., Unuma, T. & Akiyama, T. (1998) Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp, and red sea bream. *Fish. Sci*., **64,** 448–458.

Zeitoun, I.H., Halver, J.E., Ullrey, D.E. & Tack, P.I. (1973) Influence of salinity on protein requirements of rainbow trout (*Salmo gairdneri*) fingerlings. *J. Fish. Res. Board Can*., **30,** 1867–1873.

Zhou, Q., Tan, B., Mai, K. & Liu, Y. (2004) Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. *Aquaculture*, **241,** 441–451.

